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Characterizing the induction of diabetes in juvenile cynomolgus monkeys with different doses of streptozotocin

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Juvenile (2–3 years old) cynomolgus monkeys are frequently used as recipients in non-human primate islet transplantation studies. The aim of this study was to examine the effects of different doses of streptozotocin (STZ), and find the optimal dose for inducing diabetes in these monkeys. Fifteen juvenile (2–3 years old) cynomolgus monkeys were separated into three groups and administered with different doses of STZ (100, 68 or 60 mg kg^{-1}). Basal and glucose-stimulated blood glucose, insulin, and C-peptide levels, as well as body weights were monitored. Hepatic and renal function tests and pancreatic immunohistochemistry were performed before and after STZ treatment. Monkeys treated with both 100 and 68 mg kg^{-1} of STZ exhibited continuous hyperglycemia, which coincided with a nearly complete loss of islet β-cells. Two monkeys received 60 mg kg^{-1} of STZ, but only one became completely diabetic. During the first week following STZ treatment, hepatic and renal function slightly increased in these three groups. However, 24 hours post-STZ, serum total bile acid levels were significantly increased in monkeys treated with 100 mg kg^{-1} than those treated with 68 mg kg^{-1} of STZ (P<0.05). These data suggest that 100 mg kg^{-1} and 68 mg kg^{-1} of STZ can safely induce diabetes in cynomolgus monkeys aged 2–3 years, but 68 mg kg^{-1} of STZ, rather than 100 mg kg^{-1} of STZ, may be more appropriate for inducing diabetes in these monkeys. Furthermore, body surface area, rather than body weight, was a more reliable determinant of dosage, where 700 mg m^{-2} of STZ should be the lower limit for inducing diabetes in juvenile monkeys.

cynomolgus monkeys, diabetes, streptozotocin

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Insulin-dependent diabetes mellitus (IDDM) is a type of metabolic disease caused by the destruction of insulin- producing pancreatic cells. IDDM has serious effects on patients and could result in many severe complications, including end-stage renal failure, blindness, and non-traumatic amputations. Although exogenous insulin injections can save lives, they cannot cure diabetes. In recent years, pancreatic islet transplantation and stem cell therapy have proven to be promising approaches for restoring the physiological secretion of insulin in IDDM patients [1−5]. However, before these approaches can be developed into clinically viable treatments, many important issues need to be resolved, such as the limited sources of human islets for cell-based diabetes therapies, the stability of functional alpha- and beta-cell masses, the safety of stem cell therapies for diabetes mellitus [6−8]. Precise and reliable animal models of diabetes mellitus would thus be very important in resolving these problems.

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Animal models of diabetes mellitus include spontaneous and induced diabetic models. Spontaneous diabetic models are primarily rodents (e.g., non-obese diabetic mice, Biobreeding rats, Otsuka Long-Evans Tokushima Fatty rats, etc.) [9–11], and have been used in elucidating the genetics and pathophysiology of diabetes mellitus. However, the genetics, pathology, and physiology of rodents are very different from humans, suggesting that the conclusions drawn from these animal models are not fully translatable to human IDDM. Non-human primates have a close phylogenetic and immunological relationship with humans. Therefore, they would be especially useful in researching human diabetes. While there availability is limited, spontaneous diabetes has also been reported in non-human primates [12–14]. Models of induced diabetes are primarily drug-induced (e.g., streptozotocin (STZ) and alloxan) or achieved with a pancreatectomy. However, these methods have respective disadvantages. For example, the pancreatectomized animal models require a daily administration of oral pancreatic enzymes to maintain normal digestive function, which could be dangerous and complex for the handler. The beta-cell toxin, alloxan, is chemically unstable, as it has a very short half-life and rapidly decomposes into non-diabetogenic alloxanic acid in aqueous solution [15]. Additionally, the diabetogenic dose of alloxan has a narrow range, and a slight overdose may be lethal [16]. Although alloxan-induced diabetic monkeys have been reported in previous studies [17,18], alloxan is currently used to induce IDDM primarily in rodents and rabbits. STZ, derived from *Streptomyces acbromogenes*, is a commonly used diabetogenic toxin, which selectively kills insulin-producing cells, and has been widely used in IDDM induction in rodents, monkeys, and other animals. Previous studies reported that a single administration of 150 or 55 mg kg^{-1} of STZ can consistently, reliably, and safely induce IDDM in 6–8 month or 3–6 year-old cynomolgus monkeys, respectively [19,20]. Cynomolgus monkeys have been frequently used as recipients in nonhuman primate islet transplantation studies due to their ease of handling and readiness for transplantation [21–24]. However, the optimal dosage for IDDM induction in juvenile (2–3 years old) cynomolgus monkeys is still unclear. Additionally, other studies have also suggested that the genus and age of monkeys have an important effect on their sensitivity to STZ [25,26]. Therefore, in the present study, we characterized the induction of stable IDDM in juvenile cynomolgus monkeys (2–3 years old) using three different doses of STZ, in hopes of finding an optimal dose that produces minimal hepatic and renal toxicity.

1 Materials and methods

1.1 Animals

Fifteen male cynomolgus monkeys (2–3 years old; 2.7–4.1 kg), which were free of *Mycobacterium tuberculosis*, *Shigella*, *Salmonella*, Helminths, Ectoparasites, *Entamoeba histolytica* and herpes B virus, were used in this study. Animal care and use were conducted at the Primate Research Center of Wincon TheraCells Co. in Nanning, China (an AAALAC accredited facility), under the guidance of the Animal Laboratory Protocol established by the Guangxi Bureau of Science and Technology and approved by the Institutional Animal Care and Use Committee (IACUC). Monkeys were reared in individual cages in the same room with other animals. Ambient temperature in the room was kept at 25°C, and humidity was set between 40% and 70%. The air was replaced 12 times hourly. Lights were on for 12 h, from 07:30 to 19:30. All animals had a continuous water supply and were fed with the same commercially prepared monkey food, plus fruits twice daily. The animals were conditioned for a minimum of 1 month prior to the start of the experiment.

1.2 Induction of diabetes with STZ

All monkeys were fasted for 12–15 h prior to receiving STZ, which was administered under anesthesia with ketamine (10 mg kg^{-1} of body weight) and atropine (0.04 mg kg^{-1}). One-hundred mg of STZ (Sigma-aldrich, Saint Louis, MO) was dissolved into 50 μL of 0.05 mol L^{-1} citric acid (pH 4.5) and 5 mL of saline, and then diluted into 20 mL (final volume) of saline at 4°C [24]. Immediately after dissolving, 100 (n=7), 68 (n=6), and 60 (n=2) mg kg^{-1} of STZ were administered via the saphenous vein over a 5-minute period. Diabetic monkeys were treated with two injections of insulin per day to prevent metabolic dysfunction. Insulin doses were determined via previously determined scale, which is as follows: glucose levels < 11.1 mmol L^{-1} = no insulin; glucose levels between 11.1–16.6 mmol L^{-1} = 1–2 U of insulin; glucose levels between 16.6–22.2 mmol L^{-1} = 2–4 U of insulin; and glucose levels > 22.2 mmol L^{-1} = 4–6 U of insulin [19].

1.3 Metabolic studies

Blood glucose levels were monitored twice daily during the first week after the STZ injection, and twice daily every Monday and Thursday thereafter, using a Roche Glucotrend monitor. An intravenous glucose tolerance test (IVGTT) was performed one month after the STZ injection. Briefly, glucose (0.5 g kg^{-1} of body weight) was injected into the saphenous vein of the animal through a 24-gauge catheter. Blood glucose levels were recorded at 0, 3, 5, 10, 20, 30, and 60 min with a Roche Glucotrend monitor. Plasma was collected at 0, 10, 20, and 30 min for insulin and C-peptide measurements. K value, representing the rate of glucose disappearance, was calculated using the following formula: $K=69.3/t_{1/2}$, where $t_{1/2}$ equals the time required for the glucose concentration to be halved.

1.4 Blood chemistry

For hepatic and renal function tests, serum was collected before, and 24 h, 72 h, one week, and one month after STZ administration. The following parameters were measured using an automatic analyzer (7170, Hitachi, Tokyo, Japan): alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bile acid (TBA), cholinesterase (CHE), lactate dehydrogenase (LD), adenosine deaminase (ADA), blood urea nitrogen (BUN), and creatinine (Cr).

1.5 Body weight and surface area measurements

Cynomolgus monkeys were weighed before, and one and five weeks after STZ administration. Monkeys were transferred by a carrier and weighed on a weight scale. The body surface area (m^2) was calculated from the body weight (g) using the following formula: surface area=body weight$^{0.67}$× 12/10000.

1.6 Immunohistochemical staining

Normal pancreatic tissue was obtained from an autopsy from five male cynomolgus monkeys (2–3 years old). These animals were used for other experiments and were healthy at sacrifice. Diabetic pancreatic tissue was obtained from a pancreatectomy performed on the diabetic animals 2–3 months after the STZ injection. Pancreatic tissue was fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5 μm thick sections. Paraffin sections were stained using an immunofluorescent technique to visualize β- and α-cells. Briefly, the sections were dewaxed with xylene and ethanol, blocked with 1% bovine serum albumin at room temperature for 1 h, then incubated with primary antibodies overnight at 4ºC. The primary antibodies were guinea pig anti-human insulin (1:100; Zymed, San Francisco, CA) and mouse anti-human glucagon (1:1000; Sigma-Aldrich, Saint Louis, MO). The sections were then incubated with Texas red-conjugated donkey anti-guinea pig IgG or Cy2-conjugated goat anti-mouse IgG (1:200; Jackson ImmunoResearch, Baltimore, PA). Fluorescent images were captured using a Nikon 2000U microscope and SPOT RT camera. The final images were rendered via Adobe Photoshop.

1.7 Morphological examinations

The entire pancreatic area, islet area, and number of β- and α-cells were determined via immunofluorescent staining. For each pancreas, six sections (>50 μm apart) were examined.

1.8 Statistical analysis

For all comparisons, $P<0.05$ was considered statistically significant. Results were expressed as means±SD or mean (range), and groups were compared with ANOVA or non-parametric tests.

2 Results

Following the STZ injection, blood glucose levels in all groups were monitored continuously. Blood glucose levels were measured at pre-STZ injection, and 6, 20, and 28 h post-STZ injection. Corroborating previous studies [25,27], a representative triphasic blood glucose response to STZ was observed in monkeys treated with 100 mg kg^{-1} (STZ 100) and 68 mg kg^{-1} (STZ 68) of STZ (7.5 mmol L^{-1} at 6 h post-STZ, followed by 1.5 mmol L^{-1} at 20 h post-STZ, and 8 mmol L^{-1} at 28 h post-STZ). However, the insulin-dependent monkey treated with 60 mg kg^{-1} of STZ (STZ 60-ID) did not exhibit the above mentioned blood glucose response. In the STZ 60-ID monkey, there was no profound hypoglycemia detected and its blood glucose levels gradually increased to >11.1 mmol L^{-1} by day 4 post-STZ. These responses were different in other STZ-treated monkeys that required insulin to prevent ketoacidosis (STZ-ID), since their blood glucose levels were promptly augmented on the second day post-STZ. Table 1 summarizes the metabolic parameters before and after STZ treatment. Following the STZ injection, all animals in the STZ 60-ID, STZ 68, and STZ 100 groups exhibited hyperglycemia, with mean fasting blood glucose levels >22 mmol L^{-1}. There were no significant differences in the post-STZ blood glucose levels between the STZ 68 and STZ 100 groups. Additionally, the IVGTT findings suggest significant evidence of impaired carbohydrate tolerance in all three groups ($K<0.6$). Moreo-

Table 1 Metabolic assessments before and after streptozotocin administration[a)]

Group	*n*	Fasting BG (mmol L^{-1})	Non-fasting BG (mmol L^{-1})	IVGTT *K* value	IVGTT			
					Basal values of insulin (mU L^{-1})	Basal values of C-peptide (pmol L^{-1})	Peak values of insulin (mU L^{-1})	Peak values of C-peptide (pmol L^{-1})
Normal (Pre-STZ)	15	2.4±0.4	3±0.4	3.96±1.73	19(0–125)	447(180–1555)	147(36–200)	1657(624–2987)
STZ 60-NID	1	5.7	5.9	1.44	14.7	413	43.7	590
STZ 60-ID	1	26.4	28.1	0.33	1.1	28	1.9	28
STZ 68	6	22.1±4.9**	24.7±1.4**	0.34±0.08**	1.5 (0.7–3.8)**	0.4 (0–2)**	1.7 (0.8–3.8)**	3(0–15)**
STZ 100	7	26.5±3.2**	27.2±3.5**	0.37±0.22**	0.8 (0–3.2)**	8 (0–25)**	0.9 (0–3.2)**	9 (0–31)**

a) Data are expressed as mean±SD or means (range). **, $P<0.01$ vs. normal (pre-STZ).

ver, almost no circulating C-peptide and insulin could be detected after STZ treatment, and all diabetic animals in the three groups needed a subcutaneous injection of exogenous insulin (8–10 U d^{-1}) to avoid metabolic dysfunction. However, the mean fasting blood glucose level increased to only 5.7 mmol L^{-1} in the non-insulin independent monkey treated with 60 mg kg^{-1} of STZ (STZ 60-NID). IVGTT findings demonstrated that peak C-peptide levels decreased from 2582 to 590 pmol L^{-1}, and peak insulin levels decreased from 200 to 43.7 mU L^{-1}. This indicated that this monkey did not require daily exogenous insulin injections.

Immunohistochemistry revealed that, while glucagon expression was persistent in the remaining islets of both the STZ 100 and STZ 68 groups, insulin expression had disappeared (Figure 1). Morphometric analyses showed that the islet area in relation to the total pancreatic area in both the STZ 68 and STZ 100 groups decreased by 40%–50% compared with the control group. However, the difference did not reach statistical significance (P=0.055) (Figure 2A). When β-cell numbers were expressed per islet area or total pancreatic area, there was a significant decrease compared with the control group (P<0.05) (Figure 2B and C). In contrast, α-cell numbers per islet area in the STZ 68 and STZ 100 groups were significantly increased compared with the control group (P<0.05) (Figure 2D). However, α-cell numbers per total pancreatic area did not change significantly compared with the control group (P>0.05) (Figure 2E). Additionally, morphometric findings in the STZ 60-ID monkey were similar to those of the STZ 68 and STZ 100 groups. However, β-cell numbers per islet area and total pancreatic area decreased to 17% and 5%, respectively, from normal control values in the STZ 60-NID monkey. Moreover, the spatial frequency of α-cells in the diabetic and normal islets also demonstrated a distinguishable difference. In normal islets, α-cells were scattered throughout the islets, whereas in the diabetic islets, these glucagon positive cells overwhelmingly occupied the entire islet.

The findings of the hepatic and kidney function tests at various time points after an administration of 60, 68 and 100 mg kg^{-1} of STZ are presented in Table 2. Serum ALT, AST, and TBA values in STZ 100, STZ 68, and STZ 60-ID were slightly elevated within one week post-STZ. At one month post-STZ, a further increase in serum ALT, AST, and TBA values was detected. Conversely, while serum ALT, AST, and TBA values in the STZ 60-NID monkey also increased slightly at 24 to 72 h after the STZ injection, these values returned to near-normal levels within one week post-STZ. Moreover, BUN levels were slightly increased within the first 24 h, and recovered to normal within one month in STZ 100, STZ 68, and STZ 60-ID, whereas STZ 60-NID had normal kidney function after the STZ injection. No treatment-related changes were observed in other hepatic

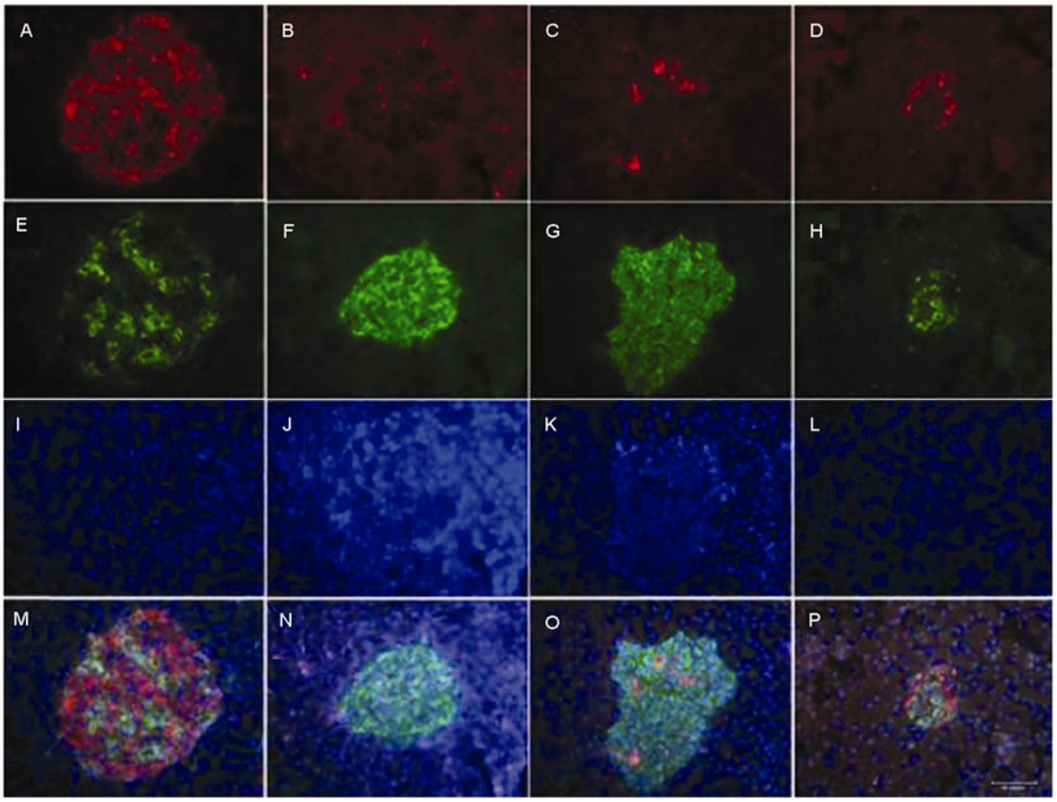

Figure 1 Immunostaining of islets from normal (A, E, I, M), STZ 100 (B, F, J, N), STZ 68 (C, G, K, O), and STZ 60-NID (D, H, L, P) monkeys. Insulin expression had disappeared, whereas glucagon expression appeared to be augmented in STZ 100 and STZ 68 groups. In contrast, there were still a few insulin-positive cells remaining in the islets of the STZ 60-NID group. Scale bar, 50 μm.

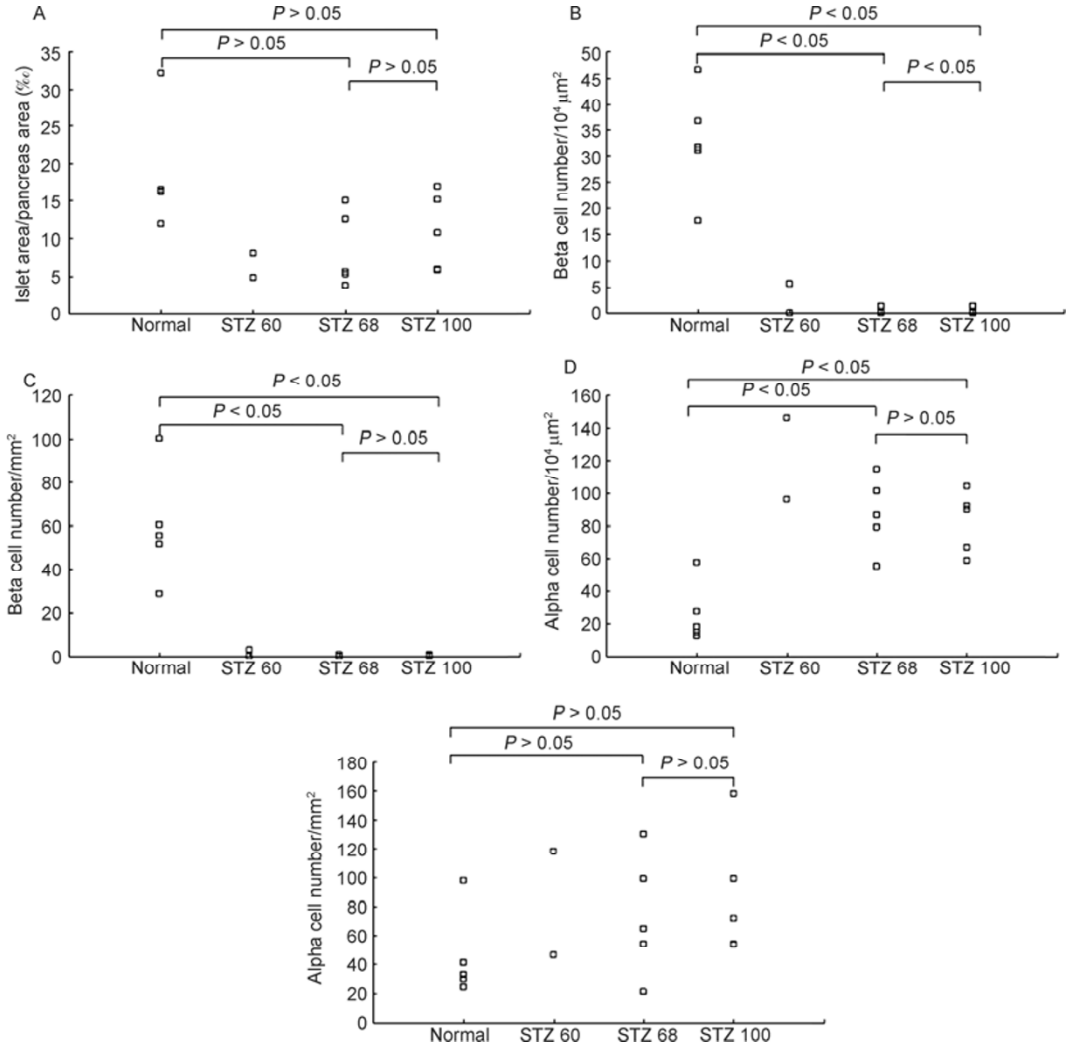

Figure 2 A, Islet area in relation to the entire pancreatic area. B, Number of β-cells per islet area. C, Number of β-cells per the entire pancreatic area. D, Number of α-cells per islet area. E, Number of α-cells per entire pancreatic area in the control group (n=5) and 60 mg kg^{-1} (n=2), 68 mg kg^{-1} (n=5), and 100 mg kg^{-1} (n=5) STZ-treated groups.

and renal parameters measured.

The body weights of all monkeys were measured at the first and fifth week after STZ administration to test whether the STZ injection would dramatically decrease their body weights in the short term, as previously described in STZ-treated mice. Figure 3A reveals that the STZ 60-ID monkey had an apparent loss in body weight (up to 15%) one week post-STZ, whereas the body weight of the STZ 60-NID monkey remained steady throughout the five weeks after the STZ treatment. The monkeys in the STZ 68 and STZ 100 groups had stable body weights during the first week post-STZ, and lost about 10% (not significant) of body weight by the fifth week post-STZ (Figure 3B and C).

3 Discussion

Animal models of IDDMs, particularly non-human primates, are important for developing therapeutic strategies for IDDM. Different STZ administration protocols have been used to induce diabetes in cynomolgus and rhesus monkeys. The findings of these studies suggest that different species and ages of monkeys respond differently to STZ treatment. Shibata *et al*. tested three different doses of STZ in adult rhesus monkeys (2.5–7.5 years old), and found that 125 mg kg^{-1} of STZ could reliably induce diabetes without any risk, whereas 100 mg kg^{-1} of STZ failed to induce IDDM and two of the seven animals died within 7 h following an administration of 150 mg kg^{-1} of STZ [25]. In another study, Thomas *et al*. found that all juvenile (2–3 years old, 3.1–3.8 kg) male rhesus macaques given 140 mg kg^{-1} of STZ developed type 1 diabetes, and this dose was not fatal [26]. These diabetic animals were maintained for >1 year, and no reversal of type 1 diabetes was observed [26]. Koulmanda *et al*. examined the effect of low versus high doses of STZ in cynomolgus monkeys (3–6 years old, 3.1–9 kg) to deter-

mine the optimal dose necessary to reliably induce diabetes with minimal toxicity. It was found that 55 mg kg^{-1} of STZ successfully induced diabetes in cynomolgus monkeys with minimal hepatic and renal toxicity, whereas animals treated with higher doses were found to have hepatic and renal damage [20]. Conversely, Thieriault *et al*. showed that 150 mg kg^{-1} of STZ consistently induced diabetes in juvenile cynomolgus monkeys (6–8 months old, 1–2 kg) with minimal morbidity and no fatalities [19].

We examined the diabetogenic and adverse effects of STZ in juvenile cynomolgus monkeys (2–3 years old, 2.7–4.1 kg) using three different doses (60, 68, and 100 mg kg^{-1}) to also determine the optimal dose for inducing diabetes. Various parameters that are associated with a diabetic states, including blood glucose, plasma insulin and C-peptide, and hepatic and kidney functioning, were monitored prior to and after inducing diabetes in these animals. Our findings revealed that 68 and 100 mg kg^{-1} of STZ successfully induce a stable diabetic state, and one out of two animals receiving 60 mg kg^{-1} of STZ developed hyperglycemia. All STZ-induced diabetic animals in STZ 100, STZ 68, and STZ 60-ID groups maintained hyperglycemia for >8 months, and no reversal of hyperglycemia was observed (Figure 4). Blood glucose levels were consistent with the pancreatic immunohistochemistry findings, where there was a nearly complete loss of β-cells in all

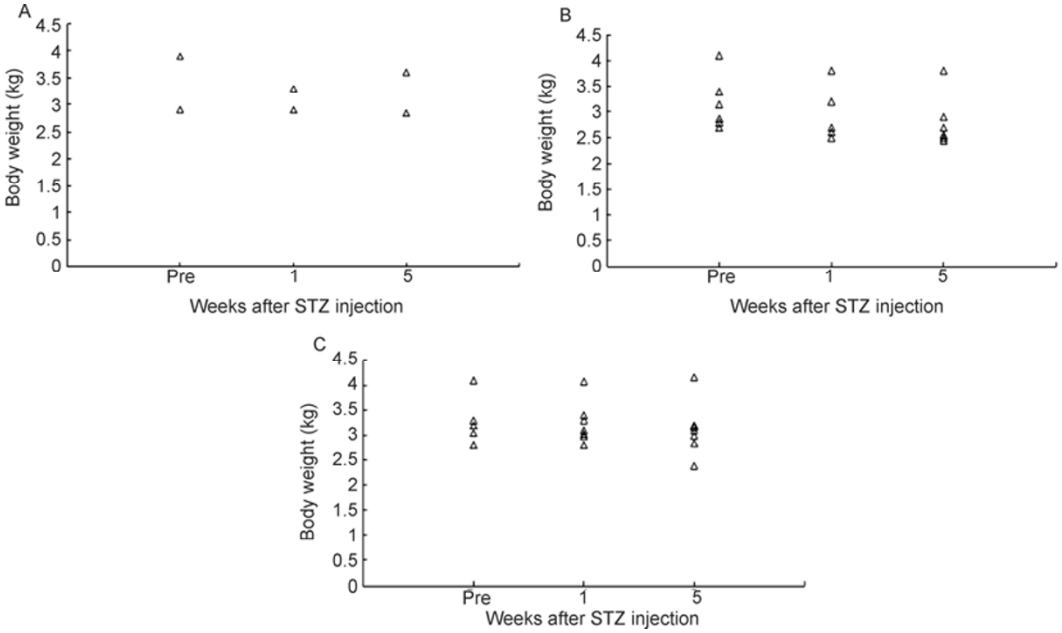

Figure 3 Body weights before, and one and five weeks after STZ treatment. A, 60 mg kg^{-1}. B, 68 mg kg^{-1}. C, 100 mg kg^{-1} groups. STZ 60-ID monkeys presented with an apparent loss in body weight (up to 15%) in the first week post-STZ, whereas STZ 60-NID, STZ 68, and STZ 100 monkeys did not demonstrate any changes in body weight in the first week post-STZ. With the exception of STZ 60-NID, by week 5 post-STZ, the body weights of all monkeys decreased by 10% compared with pre-STZ body weights, although the difference was not significant (P>0.05).

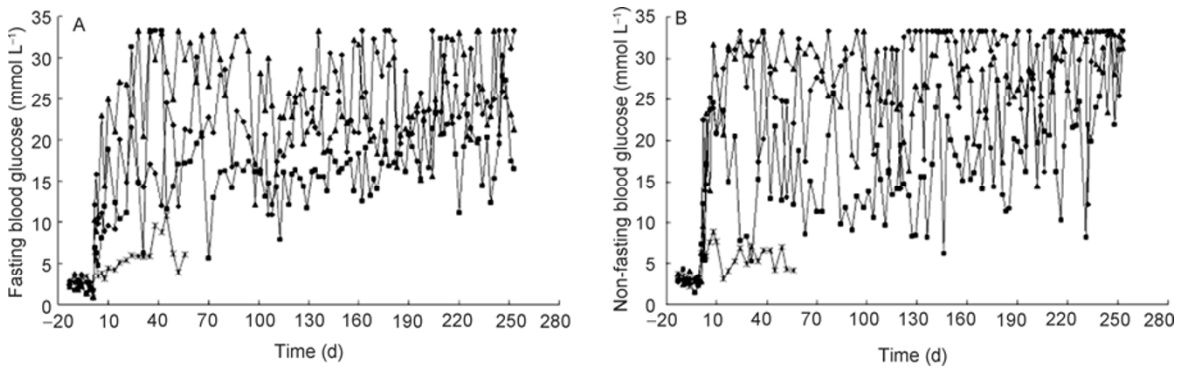

Figure 4 Fasting and non-fasting glucose levels in a representative monkey from each group, STZ 100 (■), STZ 68 (◆), STZ 60-ID (▲), and STZ 60-NID (*) are shown in panels A and B, respectively. All monkeys that received 100 and 68 mg kg^{-1} STZ exhibited hyperglycemia within two days of injection, and the fasting and non-fasting blood glucose levels increased to >11.1 mmol L^{-1}. Furthermore, there was no reversal of hyperglycemia observed throughout the long-term follow-up study. However, blood glucose levels of the STZ 60-ID monkey did not increase to >11.1 mmol L^{-1} until day 5 post-STZ, and the STZ 60-NID monkey never developed hyperglycemia.

Table 2 Hepatic and renal function before and after streptozotocin administration[a)]

Group			Baseline	Time after streptozotocin administration			
				24 h	72 h	1 week	1 month
STZ 60	ALT	mean	34	63	61	88	89
(*n*=2)	(U L^{-1})	range	(27,41)	(52,73)	(55,66)	(78,98)	(37,140)
STZ 68		mean	35	62*	69*	87*	199**
(*n*=6)		range	(16–50)	(40–84)	(52–81)	(57–156)	(74–534)
STZ 100		mean	26	40	54	46	120**
(*n*=7)		range	(7–51)	(14–100)	(24–114)	(37–67)	(36–314)
STZ 60	AST	mean	38.5	65.5	39	51	85
(*n*=2)	(U L^{-1})	range	(30,47)	(48,83)	(36,42)	(44,58)	(28,141)
STZ 68		mean	39	58*	41	54	160**
(*n*=6)		range	(34–48)	(45–72)	(31–63)	(33–84)	(45–357)
STZ 100		mean	41	63	77*	61	134*
(*n*=7)		range	(22–72)	(27–115)	(47–167)	(33–94)	(41–225)
STZ 60	TBA	mean	3	7.4	4.3	4.4	11.5
(*n*=2)	(μmol L^{-1})	range	(3,–)	(7.4,–)	(4.3,–)	(4.4,–)	(11.5,–)
STZ 68		mean	3	12.5*	10.7	5.9*	23.5**
(*n*=6)		range	(1.8–4.8)	(2-20.2)	(0.4-19.9)	(2.7-8.5)	(10.1–33.9)
STZ 100		mean	2.2	40.6**†	32.4**	13	21.8**
(*n*=7)		range	(0.2–8.3)	(4.6–72.6)	(1.3–84.6)	(0.1–42.8)	(6.9–65.5)
STZ 60	CHE	mean	10416	10757	11879	11742	16194
(*n*=2)	(U L^{-1})	range	(9129,11703)	(9051,12462)	(9743,14014)	(10219,13265)	(11081,21306)
STZ 68		mean	11239	11875	12795	14733*	14879*
(*n*=6)		range	(9211–14138)	(10034–14804)	(10701–17145)	(12409–19562)	(12246–21093)
STZ 100		mean	12499	12927	13584	17474	18060
(*n*=7)		range	(6464–20879)	(6664–19881)	(7318–20361)	(11562–21935)	(11100–23816)
STZ 60	LD	mean	316	459	414	495	300
(*n*=2)	(U L^{-1})	range	(286,346)	(541,377)	(378,449)	(404,585)	(289,310)
STZ 68		mean	355	624	472	404	515
(*n*=6)		range	(218–512)	(332–1383)	(250–1100)	(261–630)	(199–1153)
STZ 100		mean	445	496	409	392	355
(*n*=7)		range	(225–1208)	(295–1204)	(326–529)	(363–454)	(198–682)
STZ 60	ADA	mean	24	28	22	20	29
(*n*=2)	(U L^{-1})	range	(17,31)	(20,35)	(16,27)	(15,25)	(19,39)
STZ 68		mean	26	29	25	23	27
(*n*=6)		range	(15–42)	(20–49)	(13–43)	(10–39)	(17–38)
STZ 100		mean	27	26	26	24	27
(*n*=7)		range	(19–38)	(18–42)	(19–46)	(17–33)	(17–43)
STZ 60	BUN	mean	7.52	7.5	7.51	11.75	11.59
(*n*=2)	(mmol L^{-1})	range	(8.77,6.26)	(7.42,7.57)	(4.07,10.94)	(4.45,19.05)	(7.4,15.78)
STZ 68		mean	7.22	9.95*	8.32	9.13	8.89
(*n*=6)		range	(5.34–9.5)	(7.48–12.65)	(7.63–9.3)	(6.59–14.39)	(4.69–13.53)
STZ 100		mean	6.68	8.62*	8.56	9.21	9.04*
(*n*=7)		range	(5.41–8.32)	(5.96–12.65)	(6.61–10.99)	(5.5–14.06)	(6.22–11)
STZ 60	Cr	mean	58	54	66	92	53
(*n*=2)	(μmol L^{-1})	range	(53,63)	(51,56)	(55,77)	(61,123)	(67,39)
STZ 68		mean	58	60	61	60	40**
(*n*=6)		range	(53–64)	(54–70)	(44–75)	(42–90)	(31–49)
STZ 100		mean	68	66	63	80	62
(*n*=7)		range	(56–82)	(49–87)	(50–74)	(44–132)	(25–123)

a) Data are expressed as means (range). *, $P<0.05$ vs. basal; **, $P<0.01$ vs. basal; †, $P<0.05$ vs. STZ 68.

diabetic animals after STZ treatment. However, while the STZ 60-NID monkey never became diabetic, the number of β-cells decreased to 5% of the normal control after STZ treatment. This finding suggests that the compensatory activation of the remaining β-cells may lead to the production of sufficient amounts of insulin. Thus, a >95% reduction in the number of β-cells may be necessary for the onset of hyperglycemia in cynomolgus monkeys that are 2–3

years of age.

While Koulmanda *et al*. reported that 55 mg kg^{-1} of STZ was sufficient to consistently induce diabetes in cynomolgus monkeys [20], our results showed that one of two monkeys receiving 60 mg kg^{-1} of STZ failed to lose all β-cell function and subsequently become diabetic. The reason for this discrepancy may be the age of the monkeys. Koulmanda *et al*. used older monkeys, which were 3–6 years old, whereas we used younger monkeys, which were 2–3 years old. The islets of younger non-human primates may be more resistant to destruction, and thereby may influence the dose of STZ required to induce diabetes. However, it is still unclear why only one of the two monkeys of the same age became diabetic after receiving 60 mg kg^{-1} of STZ. We speculate that different body weights (2.9 and 3.9 kg) may be a reason for this dichotomy. Wijkstrom *et al*. suggested that the dose of STZ depends on body surface area rather than body weight [28]. We found that the dosages were different on an mg m^{-2} basis between the STZ 60-NID and STZ 60-ID monkeys, although both received the same dose (60 mg kg^{-1}) on an mg kg^{-1} basis (Table 3). Additionally, by extrapolating our data, it was found that a dose >760 mg m^{-2} of STZ may reliably induce IDDM in cynomolgus monkeys 2–3 years old of age, whereas a dose <700 mg m^{-2} of STZ is not effective.

The adverse effects of STZ mainly include hepatic and renal injury. Although the indices of hepatic and renal function (i.e., ALT, AST, TBA, and BUN) in the STZ 68 and STZ 100 groups were slightly elevated within one week post-STZ, the above indexes were still in the near-normal range. Our results differ from those of Koulmanda *et al*., who reported that 100 mg kg^{-1} of STZ markedly elevated liver and renal function values in monkeys post-STZ. One reason for this may be that younger animals have a higher threshold of sensitivity to STZ, as the previous studies described [19]. In addition, most of the 2–3 year-old monkeys used in our study had stable body weight post-STZ, whereas Koulmanda *et al*. reported a rapid loss in body weight of up to 20% in the 3–6 year-old monkeys receiving either a high or low dose of STZ. These findings further suggest that younger monkeys have a more stable metabolism and resistance to STZ than older monkeys. One month post-STZ, the ALT, AST, and TBA levels were further increased, likely due to the adverse effects of hyperglycemia than the direct damage induced by STZ. Thus, diabetic models can be reliably induced in juvenile cynomolgus monkeys (2–3 years old) with either 68 or 100 mg kg^{-1} of STZ without marked hepatic or renal impairment. However, we also noted that serum TBA levels had significantly increased in the STZ 100 than STZ 68 group at 24 h after the STZ injection. However, there were no significant differences in serum ALT and AST between the two groups (Table 2). This suggests that 100 mg kg^{-1} of STZ in 2–3 year-old cynomolgus monkeys may result in slightly more severe hepatic damage than 68 mg kg^{-1} of STZ, since serum TBA is more sensitive than AST in determining the severity of liver disease [29].

In conclusion, juvenile cynomolgus monkeys (2–3 years old) are more tolerant to STZ than older cynomolgus monkeys of previous studies. Therefore, diabetes can be induced in these monkeys with either a 68 or 100 mg kg^{-1} STZ injection without any marked hepatic or renal impairment, but a low dose of STZ (68 mg kg^{-1}) may be more appropriate. Less than 60 mg kg^{-1} of STZ does not appear to reliably induce diabetes in 2–3 year-old cynomolgus monkeys. Furthermore, STZ dosing should be based on body surface area rather than body weight, with 700 mg m^{-2} of STZ being the lower limit for inducing IDDM in 2–3 year-old cynomolgus monkeys.

Table 3 Comparison of streptozotocin dosing using body weight versus body surface area

Group	Animal ID	BW (kg)	STZ (mg kg^{-1})	Total STZ (mg)	Surface area (m^2)	STZ (mg m^{-2})
STZ 60-ID	0511971	3.9	60	234	0.3056	766
STZ 60-NID	0202673	2.9	60	174	0.2506	694
	0301001	3.38	68	229.8	0.2777	827.7
	0302001	4.1	68	278.8	0.316	882.3
STZ 68	0504411	2.86	68	194.4	0.2483	783.2
	0504409	2.8	68	190.4	0.2448	777.8
	0503008	2.7	68	183.6	0.2389	768.5
	0504425	3.15	68	214.2	0.2649	808.6
	0201077	3.3	100	330	0.2733	1207.5
	0208529	3.2	100	320	0.2677	1195.4
	0101535	3.2	100	320	0.2677	1195.4
STZ 100	0012041	4.1	100	410	0.316	1297.5
	0012033	3.05	100	305	0.2592	1176.7
	0108127	3.3	100	330	0.2733	1207.5
	0401739	2.8	100	280	0.2448	1143.8

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